## **Supporting Information**

## Multi organoids-on-a-chip from hiPSCs for assessment of prenatal

## antidepressant clomipramine

Fangchao Yin, <sup>a,d,1</sup> Xu Zhang, <sup>a,1</sup> Li Wang, <sup>e</sup> Yaqing Wang, <sup>a,d</sup> Yujuan Zhu, <sup>a,d</sup>

Zhongyu Li, a Tingting Tao, a,d Wenwen Chen, a,d Hao Yua and Jianhua Qin\*a,b,c,d

<sup>a</sup> CAS Key Laboratory of SSAC, Department of biotechnology, Dalian Institute of Chemical Physics, Chinese Academy of Sciences.

<sup>b</sup> Institute for Stem Cell and Regeneration, Chinese Academy of Sciences, Beijing, China.

<sup>c</sup> CAS Center for Excellence in Brain Science and Intelligence Technology, Chinese Academy of Sciences, Shanghai, China.

<sup>d</sup> University of Chinese Academy of Sciences, Beijing, China.

<sup>e</sup> Institutes of Biomedical Sciences, Fudan University, Shanghai, 200032, China.

<sup>1</sup>These authors contribute equally to the work.

\* Corresponding author: Prof. J. Qin, E-mail: jhqin@dicp.ac.cn

**Table S1.** Lists of antibodies used for immunostaining.

Primary antibodies	Supplier	Reference	Species	Dilution
albumin	Bethyl	A80-129A	Goat	1:1000
CYP3A4	Absin	abs132219	Mouse	1:500
active caspase 3	Abcam	ab32042	Rabbit	1:250
cardiac troponin T	Thermo	MA5-12960	Rabbit	1:200

Secondary antibodies	Supplier	Reference	Dilution
Cy3-labeled Donkey Anti-Goat lgG	Beyotime	A0502	1:50
(H+L)			
Anti-rabbit lgG (H+L), F(ab')2 Fragment	Cell Signaling	4412	1:500
(Alexa Fluor <sup>R</sup> 488 Conjugate)			
Anti-rabbit lgG (H+L), F(ab')2 Fragment	Cell Signaling	8889	1:500
(Alexa Fluor <sup>R</sup> 594 Conjugate)			
Anti-mouse lgG (H+L), F(ab')2 Fragment	Cell Signaling	4408	1:500
(Alexa Fluor <sup>R</sup> 488 Conjugate)			
Anti-mouse lgG (H+L),	Cell Signaling	8890	1:500
F(ab')2 Fragment (Alexa Fluor <sup>R</sup> 594			
Conjugate)			
DAPI	Cell Signaling	4083	1:10000

**Table S2.** Primer pairs used to examine mRNA expression of specific genes during the development of liver organoids.

Primer	Forward sequence (5'- 3')	Reverse sequence (5'- 3')	
NANOG	GATTTGTGGGCCTGAAGAAA	CTTTGGGACTGGTGGAAGAA	
OCT4	GGAGAAGCTGGAGCAAAACC	TGGCTGAATACCTTCCCAAA	
SOX17	GTGGACCGCACGGAATTTG	GGAGATTCACACCGGAGTCA	
FOXA2	CGACTGGAGCAGCTACTATGC	TACGTGTTCATGCCGTTCAT	
AFP	CTTTGGGCTGCTCGCTATGA	GCATGTTGATTTAACAAGCTGCT	
ALB	GCCTTTGCTCAGTATCTT	AGGTTTGGGTTGTCATCT	
PXR	AAGCCCAGTGTCAACGCAG	GGGTCTTCCGGGTGATCTC	
HNF-α			
CYP1A2	GCCATTAACAAGCCCTTGAG	ATGGCCAGGAAGAGGAAGAT	
CYP2D6	CCTTCGCCAACCACTCC	GCAGAAAGCCCGACTCCT	
CYP2C19	CGGATTTGTGTGGGGAGAGGG	GCAAATCCATTGACAACAGGAGTT	
CYP3A4	TTCAGCAAGAAGAACAAGGAC	GGTTGAAGAAGTCCTCCTAAGC	
	AA		
β-Actin	AAATCTGGCACCACACCTTC	AGAGGCGTACAGGGATAGCA	



Figure S1. Characterization of hiPSCs-derived cardiac organoids a, Bright field images of cardiac differentiation from day 0 to day 9. Scale bars: 200  $\mu$ m. b, Examination of the pluripotency markers OCT4A and NANOG at 0 and 20 days of differentiation. The expression values were normalized to the  $\beta$ -actin expression level. n = 3 replicates. Data are shown as mean  $\pm$  SD. \*\*, P-value <0.01.



Figure S2, Characterization of hiPSCs-derived liver organoids a, The representative bright field

images of the spheroids were obtained at different stages of liver organoid generation. Scale bars: 200  $\mu$ m. **b-c**, mRNA expression of pluripotent markers OCT4 and NANOG (**b**), endodermal markers SOX17 and FOXA2 (**c**) was quantified by RT-PCR. The expression values were normalized to the  $\beta$ -actin expression level. n = 3 replicates. Data are shown as mean  $\pm$  SD. \*\*, P-value < 0.001 \*\*\*, P-value < 0.001.



Figure S3. MS detection of clomipramine before and after liver metabolism. The peak of desmethylclomipramine (m/z 301) was observed only after liver metabolism.